BURST ANALYSIS OF CHEMICALLY STIMULATED SPINAL CORD NEURONAL NETWORKS CULTURED ON MICROELECTRODE ARRAYS

M. Grattarola[1], M. Chiappalone[1], F. Davide[2], S. Martinoia[1], M.(B.) Tedesco[1], N. Rosso[1], A. Vato[1]
[1]Neural and Bioelectronic Technologies Group, Department of Biophysical and Electronic Engineering, University of Genoa, Italy,
[2]Telecom Italia S.p.A., Rome, Italy

Abstract- **Planar microelectrode arrays allow the recording of the electrophysiological activity of cultured neurons for long time periods and from different sites of the network.**
In the developing Central Nervous System, several neural networks are spontaneously active and show a typical high frequency activity pattern called burst. Such behavior seems to play an important role in subsequent maturation.
Here we analyze how the bursting electrophysiological activity of cultured spinal cord neurons (14 – 18 DIV) from the chick embryo is affected by cyclothiazide (CTZ) which acts on AMPA receptors.
Changes in the patterns of electrophysiological activity are described in detail.

Keywords **- microelectrode array, spinal cord network, burst activity.**

I. INTRODUCTION

Nowadays, arrays of planar and 3-dimensional microelectrodes [1] are becoming a reliable tool in the framework of in vitro electrophysiology, including dissociated neurons [2],[3], acute brain slices and organotypic brain slices.

Two main features make microelectrode arrays (MEAs) a valuable tool for electrophysiology, namely: a) they are non-invasive and therefore, under appropriate conditions, they can register the electrophysiological activity of neurons for a long period of time (i.e., from several minutes up to several hours). b) They allow a multi-site recording [4]. In this paper, by taking advantages of these features we analyze the signals generated by neurons dissociated from the embryo spinal cord in terms of electrophysiological activity. We recorded from 8 electrodes simultaneously; in this way is possible to analyze the variation induced by the drug and the correlation between different channels (i.e., different area in the neural network) with respect to the chemical stimuli.

Cultured spinal neurons from the chick embryo were chosen as a neurobiological system quite appropriate for long-term recording. It is well known that neuronal networks in the developing spinal cord are spontaneously active. Evidence is also accumulating that such activity plays an important role in the maturation of the networks [5].

Electrophysiological rhythmic activity mostly appears in the form of "bursting" and the interval among bursts is in the scale of seconds or even minutes. A burst is characterized by a rapid sequence of several spikes separated from each other by a few ms. In other words, from the point of view of the signal patterns, we deal with time sequences where episodes in the range of seconds (bursts) are separated by periods of silence in the tens of seconds range (InterBurst interval, IBI).

It should be underlined that, because of a number of sources of biological variability, the signals resulting from one specific experiment show a similar pattern but not a one to one reproduction of other experiments.

II. METHODOLOGY

Dissociated neurons were obtained from the spinal cord of chick embryos after 7 – 8 days of incubation, according to a modified version of the protocol described in [6]. Neurons were seeded on microelectrode arrays covered with adhesion promoting molecules (Polylisyne, laminin). Electrophysiological signals were recorded after 14 –18 days in vitro (DIV), to allow the formation of synaptic contacts among the cells.

A. Experimental protocol

In order to investigate the role of AMPA receptor in the developing Central Nervous System, a particular drug, cyclothiazide (CTZ), was used. CTZ acts blocking AMPA receptor desensitization.

The experimental protocol consisted of about 18 minutes of acquisition in each of the two different situations:

1) Control condition, corresponding to the spontaneous activity in culture medium (NeuroBasal medium, Sigma).
2) Cyclothiazide treatment, added at a final concentration of 30 μM.

We repeated this procedure twice, during the experiments that, from now on, we will call EXP1 (cell culture of 14 DIV), and EXP2 (cell culture of 18 DIV) .

B. Microelectrode Array and measurement system

The array used for our experiments [7] is made of glass with 60 gold microelectrodes, 40 of which used for acquisition (10 micron diameter). The 40 recording electrodes were subdivided into 5 groups of 8 electrodes each, directly connected with the amplifier via an 8 poles shielded cable.

An experimental set up, based on the microelectrode array and constituted by the following functional elements, was developed :

1) Microelectrode array, which is itself an interface between the biological and the electrical environment;
2) Faraday Cage, to avoid electromagnetic interference;

Report Documentation Page

Report Date	Report Type	Dates Covered (from... to)
25 Oct 2001	N/A	-

Title and Subtitle Burst Analysis of Chemically Stimulated Spinal Cord Neuronal Networks Cultured on Microelectrode Arrays	**Contract Number**
	Grant Number
	Program Element Number
Author(s)	**Project Number**
	Task Number
	Work Unit Number
Performing Organization Name(s) and Address(es) Neural and Bioelectronic Technologies Group Department of Biophysical and Electronic Engrg Univesrity of Genoa, Italy	**Performing Organization Report Number**
Sponsoring/Monitoring Agency Name(s) and Address(es) US Army Research, Development & Standardization Group (UK) PSC 802 Box 15 FPO AE 09499-1500	**Sponsor/Monitor's Acronym(s)**
	Sponsor/Monitor's Report Number(s)

Distribution/Availability Statement
Approved for public release, distribution unlimited

Supplementary Notes
Papers from 23rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society, October 25-28, 2001, held in Istanbul, Turkey. See also ADM001351 for entire conference on cd-rom.

Abstract

Subject Terms

Report Classification unclassified	**Classification of this page** unclassified
Classification of Abstract unclassified	**Limitation of Abstract** UU

Number of Pages
4

3) 8 channels amplifier and filtering stage (gain=100);
4) Long term acquisition instrumentation: Digital Tape Recorder (BioLogic DTR-1802) with a maximum of 8 recording channels at the sampling frequency of 12kHz and GPIB Interface. Each channel is connected to the MEA via the amplification and filtering system. For an easier identification, we named them according to the labels on the DTR display: L1, L2, L3, L4, R1, R2, R3, R4.
5) Oscilloscope for real time monitoring of signals;
6) PC for off-line data management.

C. Signal Processing

Signals collected from a microelectrode array have typical amplitudes in the range 0.1-0.4 mV and are embedded in biological and thermal noise ranging from 5μV up to 10μV peak to peak. DTR was used with a sampling frequency of 12kHz with a resolution of 2 bytes per sample. This resulted in a large amount of data: about 240 MB for one experimental phase of 20 min acquisition, which are difficult to be processed with general-purpose commercially available processing tools. For this reason, custom software tools for data management and signal processing were developed.

Data formats were defined for both raw data and post-processed data and standard routines for graphical representation were implemented. The post-processed data are ascii file compatible with Microsoft® Excel or Microcal(™) Origin® format.

Statistical analysis together with peak detection pre-processing techniques were developed.

Peak Detection The algorithm uses a floating window and a peak-to-peak threshold (absolute threshold). A mask is shifted along the raw signal and when a spike is detected the feature related to the signal is saved (see Fig. 2).

The Spike parameters are:
1) Window time length, set at 5 msec
2) Threshold value, set at 0.12 mV p-p

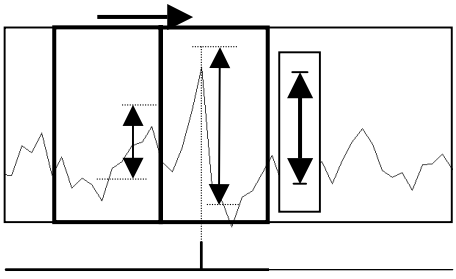

Fig. 1: Peak Detection Algorithm by means of a floating window and absolute threshold

The Peak detection produces an output file containing the following parameters:
- Time occurrence of single spike (ms)
- Amplitude peak-to-peak of spikes (mV)

Burst Detection The spontaneous activity obtained from cultured spinal networks ranges from apparent stochastic spiking to organized bursting, a condition which denotes synchronous activity of the cells. The description of network activity at burst level, analyzing the burst pattern in different pharmacological conditions, provides a vast amount of information about network behavior without having to deal with the complexities of spike analysis [8].

To investigate burst patterns, we developed an algorithm for their automatic detection, utilizing pre-processed data by the peak-detection algorithm. Data coming from the peak-detection are greatly reduced with respect to their complexity and to their original memory occupancy. A window is shifted along the processed signal and when a spike cluster is detected a graph is shown to the user. This graph represents the time of occurrence of spike clusters and their amplitudes in arbitrary units, which represent the sum of spikes amplitudes that belong to the same cluster. Before having a real burst detection, it is necessary to define a threshold level, which eliminates the spike clusters that cannot be considered as bursts.

The Burst parameters that we imposed are:
1) Window time length, set at 650 msec
2) Threshold level, set at 3500 units.

The extracted features for each burst detected, saved on an Excel compatible file, are the following:
- Time of occurrence (min)
- Burst Duration (msec)
- InterBurst Interval – IBI (ms), defined as the time length between the end of a burst and the beginning of the next one.
- Burst amplitude (arbitrary units)

Once that bursts have been detected, it is also possible to calculate, following the algorithms implemented for single spike analysis, the Joint Inter Burst Interval (J-IBI) and the Cross – conditioned Inter Burst Interval (C-IBI), which hold the same definition as for the J-ISI and C-ISI [3,8], provided that now the event is the burst occurrence instead of the spike occurrence.

III. Results and Discussion

Fig. 2 shows a spontaneous activity interval of about 10 minutes recorded from channel L1 during EXP1. Simply by eye inspection, it is possible to identify "evident" bursts (circle) and signals which look like single isolated events (arrow). It is not easy to discriminate among these signals on the basis of different neurobiological meanings.

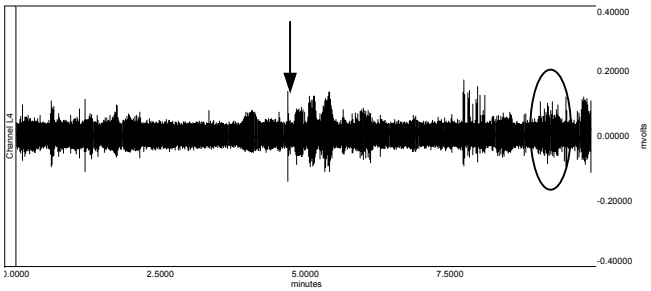

Fig.2: Spontaneous activity recorded from one microelectrode of the array during EXP1: the circle holds an evident burst, the arrow indicates a single isolated event.

The activity pattern in basal condition is about the same for all the channels and during both EXP1 and EXP2.

During the second experimental phase, the spinal neurons respond to the presence of CTZ with significantly different signals, in which burst frequency seems to be higher than in case of spontaneous activity, as shown in Fig. 3.

This is an expected behavior, because the blockade of AMPA receptor desensitization by cyclothiazide potentiates the responses of spinal cord neurones [9] and therefore makes the synchronized activity much more evident.

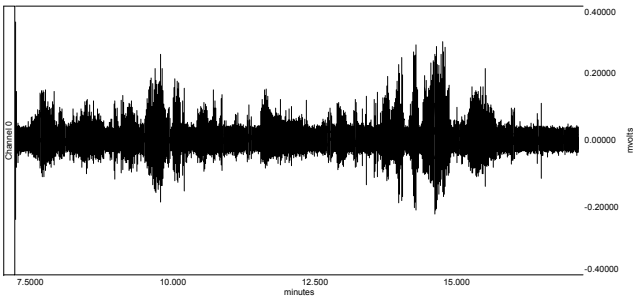

Fig.3: Electrophysiological activity recorded from one microelectrode of the array during EXP1, after CTZ treatment. Note how, only by visual inspection, there is an increasing of burst number respect the previous phase.

Bursts were selected according to Burst Detection algorithm, and both IBI and Burst duration were calculated for each recording channel.

In Fig.4 a bar graph shows the IBI mean value and the standard error for each channel during the two experimental phases of EXP1. For each channel the presence of CTZ causes a decreasing of the mean IBI, which means an improving of the electrophysiological bursting activity. At the same time, we can note a different behavior in the 8 channels, which denotes the importance of the multisite recording that only microlectrode arrays allow. For instance, in R1 and R3 the CTZ treatment has, in average, more effect than in the other channels.

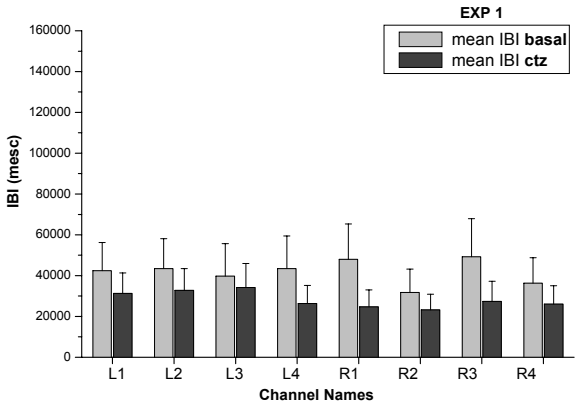

Fig. 4. Mean and standard error of IBI for each channel in EXP1. Note how mean value of IBI decreases for each channel during CTZ treatment.

In Fig.5 a bar graph shows results for EXP2, in term of IBI mean value and standard error for all the channel set in control condition and CTZ treatment. Also in EXP2 the treatment with CTZ causes an improving of bursting rate and in this case it is more evident than in EXP1.

Interestingly enough not only the increment in the bursting activity is much more evident in EXP2 in comparison to EXP1, but also the mean IBI during phase 2 (CTZ) of EXP 2 for all the channels is longer (50 sec), then in EXP 1 (30 sec). This could be due to the biological variability of the neural network preparation but also to the age of the culture (DIV14 for EXP1 and DIV18 for EXP2) which could produce more synchronized (i.e. stable) behavior of the network and an increased sensitivity to the drug action. This fact will be further analyzed in future experiments.

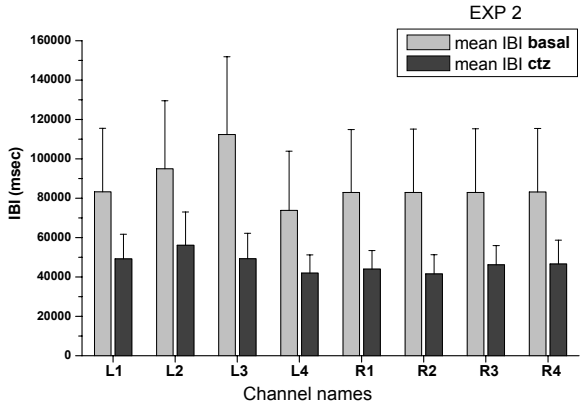

Fig. 5. Mean and standard error of IBI for each channel in EXP2. Also in this case mean value of IBI decreases for each channel during CTZ treatment.

We have also analysed the burst duration statistic values, as reported for the two experiments in TABLE I and TABLE II.

The CTZ treatment causes an increased in the standard deviation value for burst duration, both in EXP1 and EXP2, showing there is more variability of this parameter in the second phase.

Moreover in EXP 2 std values are, in general, less than mean burst duration (only one case is different, channel R2), while in EXP1 we find very high values of std, always greater than the mean burst duration (except for L3 where, however, there is a very small difference). This may be due to the fact that in EXP 2 we studied an older culture, which shows higher stability, and probably the variability due to CTZ has minor effects on the electrophysiological responses of the whole network.

TABLE I
BURST DURATION VARIABILITY EXP1

	Control	CTZ
Channel Name	mean ± std (msec)	mean ± std (msec)
L1	5289±5571	6380±7475
L2	5002±3199	6500±8020
L3	6347±3608	6798±6687
L4	4426±3766	4903±6169
R1	5679±3789	5850±7137
R2	5357±4110	4749±5442
R3	6295±3900	6298±6870
R4	5486±4409	6435±7712

TABLE II
BURST DURATION VARIABILITY EXP2

	Control	CTZ
Channel Name	mean ± std (msec)	mean ± std (msec)
L1	6211±3650	6158±4971
L2	6825±4340	6041±5070
L3	6407±3704	5918±5104
L4	5785±3845	5407±4680
R1	6428±3446	5695±5407
R2	6500±4538	8388±12042
R3	6428±4255	6175±5172
R4	6211±3621	6110±5045

V. CONCLUSION AND PROSPECTS

In a way, long term recording from in vitro populations of neurons provides a new kind of electrophysiological signals, which go on for minutes as in vivo experiments but at the same time are related to much simpler neurobiological systems. Contrary to in vivo systems, "spontaneous" (i.e. non stimulus-induced) activity is easily recorded and the effect of drugs can be tested almost immediately. With specific reference to networks of spinal neurons, signal processing confirms that this neurobiological system is able, in the presence of CTZ, to show a new kind of electrophisyological activity. More detailed analysis including conditioned bursting activity and cross-correleted activity between different channels (i.e., between well identified different region of the neuronal network) will be undertaken in the future. Also the different behavior obtained from neuronal population of different ages will be analyzed on a larger and statistically soundly basis.

ACKNOWLEDGMENT

Work supported by the National research Council of Italy (CNR) , Target project "Biotechnology" and by Telecom Italia S.p.A., protocol 0009989-01

REFERENCES

[1] G. W. Gross: "Simultaneous single unit recording in vitro with a photoetched laser deinsulated glod multielectrode surface", IEEE Trans. Biomed. Eng., BME-26, 1979, pp. 273 - 279

[2] M. Bove, M. Grattarola, S. Martinioa, G. Verreschi: "Interfacing cultured neurons to planar substrate microelectrodes: characterization of the neuron-to-microelectrode junction", Bioelectrochem. Bioeng., vol.38, pp. 255-265, 1995

[3] M. Bove, M. Grattarola, G. Verreschi :"In vitro 2D networks of neurons characterized by processing the signals recorded with a planar microtransducer array " IEEE Trans. Biomed. Eng, BME - 44, 1997, pp. 964 – 977

[4] Y. Jimbo, A. Kawana, "Electrical stimulation and recording from cultured neurons using a planar array", Bioelectrochem. Bioeng.,vol. 40, pp. 193-204, 1992

[5] RD Fields, P.G. Nelson :"Activity-dependent development of the vertebrate nervous system" Int. Rev Neurobiol, 34, 1992, pp. 133–214

[6] M. Dichter, G.Fischbach: "The action potential of chick dorsal root ganglion neurons manteined in cell culture", J. Physiology, 276,1977, pp.281-298.

[7] J.R Buitenweg, W.L.C. Rutten, W.P.A. Willems, J.W van Nieuwkasteele, "Measurement of sealing resistance of cell-electrode interfaces in neuronal cultures using impedance spectroscopy", Med. Biol. Eng. Comp., 36-5**,** pp. 630-637, 1998.

[8] G. W. Gross:"Internal dynamics of randomized mammalian networks in culture", Enabling Technologies for Cultured Neural Networks", Chapter 13, Academic Press, 1994.

[9] WM Dai, J Egebjerg, JD Lambert: "Characteristics of AMPA receptor-mediated responses of cultured cortical and spinal cord neurones and their correlation to the expression of glutamate receptor subunits, GluR1-4", Br J Pharmacol 2001 Apr 8;132(8):1859-1875.